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Amino Acid-Based Synthesis and Glycosidase Inhibition of Cyclopropane-Containing Iminosugars

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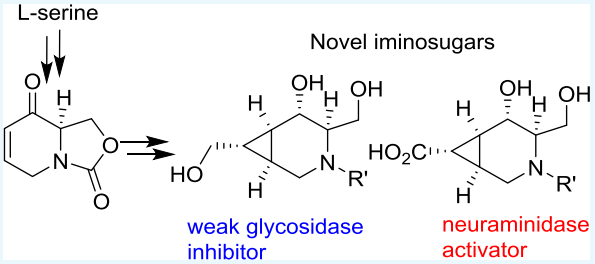
ABSTRACT: Synthesis of four iminosugars fused to a cyclopropane ring is described using L-serine as the chiral pool. The key steps are large-scale preparation of an α,β-unsaturated piperidinone followed by completely stereoselective sulfur ylide cyclopropanation. Stereochemistry of compounds has been studied by nuclear Overhauser effect spectroscopy (NOESY) experiments and ^{1}H homonuclear decoupling to measure constant couplings. The activity of these compounds against different glycosidases has been evaluated. Although inhibition activity was low (compound **8a** presents a (K_i) of 1.18 mM against β-galactosidase from *Escherichia coli*), interestingly, we found that compounds **8a** and **8b** increase the activity of neuraminidase from *Vibrio cholerae* up to 100%.



INTRODUCTION

Iminosugars are azaheterocycles with promising biological activities such as glycosidase and glycosyltransferase inhibition and modulation.[1] Many iminosugars are natural or synthetic polyhydroxylated piperidines, which can act as biomimetics of their corresponding pyranose analogues. Some of the most important natural piperidine iminosugars are nojirimycin (Figure 1, **I**) and its epimers, which, together with their deoxy analogues have turned out to be the lead molecules for drug design. Thus, stereochemical changes and functional group variation have led to iminosugars that can modulate glycosidase enzymes, exhibiting immunosuppressive, antiviral, or anti-inflammatory activities.[2] Bicyclic iminosugars, such as swainsonine (**II**), lentiginosine (**III**), castanospermine (**IV**), and their derivatives exhibit antitumor and immunosuppressive activities.[3]

Several iminosugars, miglitol (Glyset, **V**),[4] migalastat (Galafold, **VI**),[5] and miglustat (Zavesca, **VII**)[6] are commercially available for the treatment of type II diabetes and Fabry disease, and as the first oral treatment for Gaucher disease, respectively. Several other competitive inhibitors of glycosidases are being developed as new drugs and are in different phases of clinical trials.

The mechanism associated with glycosidase activity modulation is generally attributed to structural similarity to the oxacarbenium ion-like transition-state, formed during the hydrolysis of carbohydrates.[7] These transition states present diverse conformational pathways for different glycosidases,[8] making selective inhibition possible. In this context, designing conformationally restricted inhibitors seems to be an interesting approach. In addition, adequate metabolic stability is needed, which may be achieved with more rigid compounds. Recently, a study on α-mannanases showed how the enzyme surface restricts the conformational landscape of the substrate, rendering the $B_{2,5}$ conformation the most stable on-enzyme (Figure 2a).[9] In another study, a cyclopropane containing a cyclophellitol analogue, was designed as a specific β-glucosidase inhibitor for enzymes reacting through the $^4H_3$ transition-state conformation (Figure 2b).[10]

With these precedents, we expected that the introduction of a three-membered ring annulated to a piperidine ring would render novel iminosugars with a locked conformation that may be the starting point for finding the therapeutic compounds (Figure 3). The substituted cyclopropane moiety renders a fixed conformation and allows many different configurations that could increase selectivity to specific glycosidases.

The development of efficient routes for the preparation of iminosugars has received much attention from the synthetic community.[11] Most of the methods use carbohydrates as the chiral pool, which are transformed using reductive aminations[12] or other transformation strategies.[13] Alternatively, some asymmetric or biocatalysed approaches have been used.[14] But, there are fewer reports on approaches where amino acids are used as the chiral pool for the synthesis of iminosugar derivatives.[15]

Herein, we envisioned the preparation of novel bicyclic iminosugars that include the cyclopropane motif fused with piperidine starting from the natural amino acid L-serine as the

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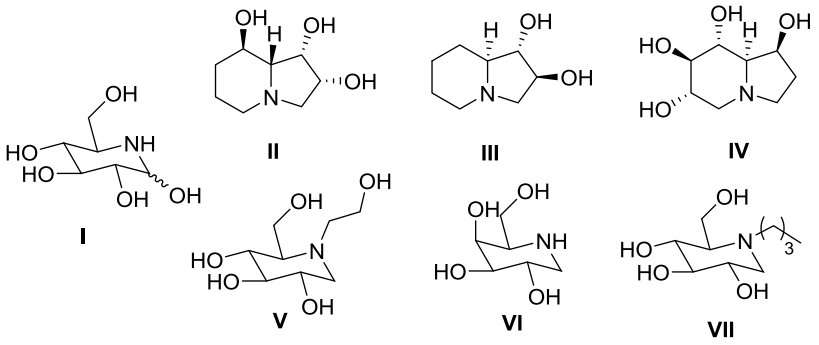


Figure 1. Structures of nojirimycin (**I**), swainsonine (**II**), lentiginosine (**III**), castanospermine (**IV**), miglitol (**V**), migalastat (**VI**), and miglustat (**VII**).

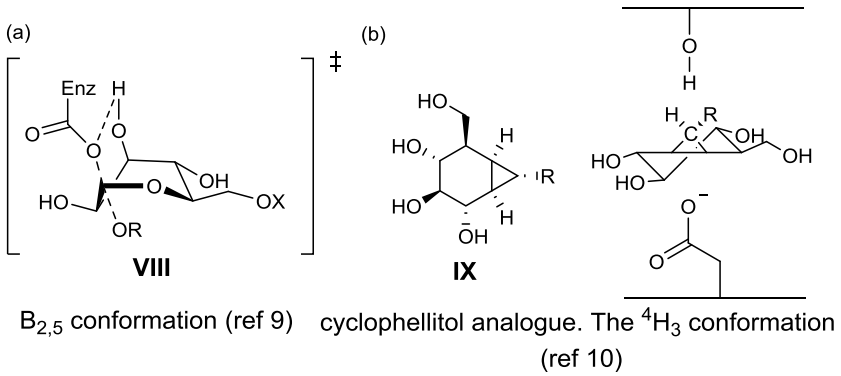


Figure 2. Transition-state conformations on-enzyme: (a) mannose $B_{2,5}$ conformation (**VIII**, reprinted with permission from ref 9) and (b) cyclophellitol analogue and its $^4H_3$ conformation (**IX**, ref 10, copyright 2017 American Chemical Society).

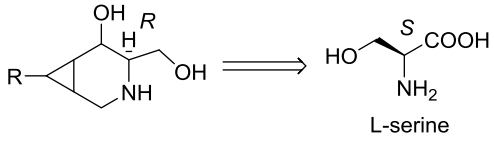


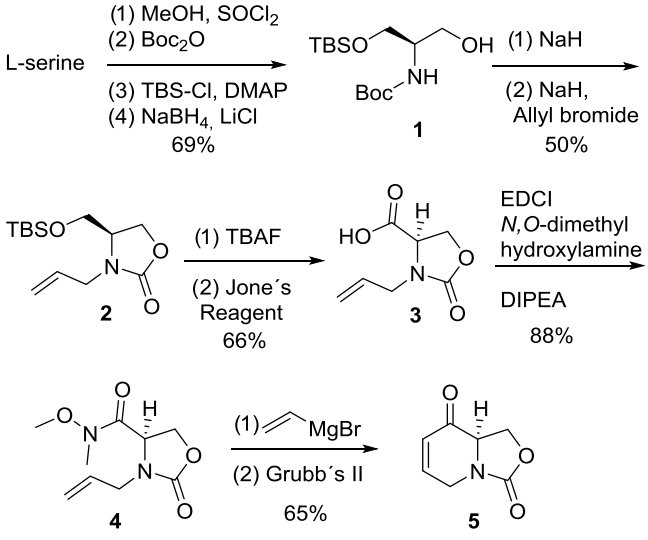
Figure 3. Structure of the target compound.

chiral pool. The final compounds present five stereogenic centers, and the synthesis involves the inversion of the configuration of the starting L-serine (*S*-configuration) into the C5 configuration of D-carbohydrates (*R*).[16] Preliminary glycosidase inhibition evaluation is shown.

RESULTS AND DISCUSSION

Our first goal was the synthesis of α,β-unsaturated ketone **5** in which the chiral center has *R* configuration. This configuration was selected as it corresponds to C5 in natural sugars and iminosugars, which share the *R* configuration in that position. This compound has already been prepared from D-serine and described.[17] In our case, we developed a synthesis approach using cheaper and natural L-serine, as depicted in Scheme 1. From this intermediate, a cyclopropanation reaction and further transformations resulted in a new family of piperidines fused to cyclopropanes. L-Serine was esterified and protected with Boc_2O, and the resulting intermediate was further protected and reduced to give desymmetrized alcohol **1** in which the configuration has changed from *S* to *R* in a few steps. This compound **1** was transformed into oxazolidinone by reaction with a base followed by allylation to give compound **2**. Following the previously reported methodology,[18] **2** was deprotected and oxidized into carboxylic acid **3**. This was converted into Weinreb amide **4**, which was treated with vinylmagnesium bromide and subjected to a ring-closing metathesis, RCM, using second-generation Grubbs' catalyst (Grubbs Catalyst M204), giving the starting material **5**.[19] This precursor containing the piperidine core was obtained in 13% global yield after 11 steps. No racemization was observed during the synthesis.

Scheme 1. Synthesis of Compound 5



The cyclopropanation reaction of **5** was performed using sulfur ylide. Interestingly, only one reaction product was observed and isolated in 70% yield. This product was designated as structure **6**, as a result of NMR analysis (nuclear

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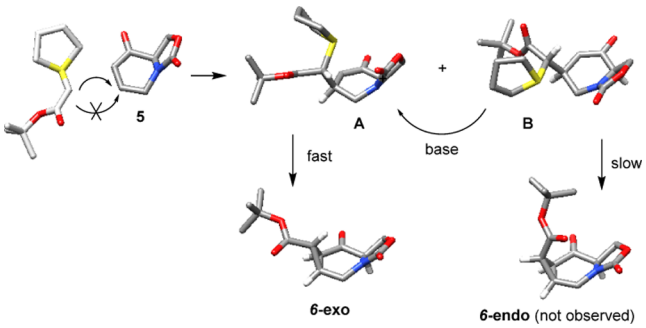
Overhauser effect (NOE) and coupling constants) of compounds **7a**−**b** (*vide infra*). Cyclopropanation occurred on the same side of oxazolidinone (endo attack) and the subsequent ring-closing step exclusively gave *exo*-cyclopropane. The ylide mediated cyclopropanation is a stepwise reaction in which the formation of the first C−C bond is the rate-determining step.[20] The attack of the ylide on **5** is more favored from the opposite face to the nitrogen lone electron pair as depicted in Scheme2; therefore, it occurs through the same face of the oxazolidinone ring (endo). This selectivity was observed previously in one unsaturated γ-lactam cyclopropanation,[21] although other precedents have described mixtures of endo and exo attacks.[22] Then, the stereoselectivity of cyclopropane is determined in the second step. Studies reported by Aggarwal's group,[23] showed that two intermediate betaines **A** and **B** are formed in a 1:1 ratio after the nucleophilic addition. The cyclization of betaine **A** is faster than that of **B**. Moreover, **B** can epimerize to give **A** before it closes the three-membered ring generally leading to high diastereoselectivity of exo-cyclopropane.

Scheme 2. Stereoselectivity of the Cyclopropanation Reaction of 5[24]



With the cyclopropane containing compound **6** in hand, the reduction of the ketone afforded a (3:2) mixture of the two diastereomers **7a**−**b** (Scheme 3). These alcohols were separated, characterized, and separately transformed into the final products. The stereochemistry of **7a** and **7b** was determined using NOE experiments and coupling constant values. Figure 4 shows the main correlations observed for **7a** and **7b** that allowed assigning the relative configuration of H6, H7, and H7a. The coupling constants between H7a, H7, and H6a were determined using homonuclear decoupling experiments. Values are shown in Figure 4, and the model agrees with the calculated angles for these couplings.

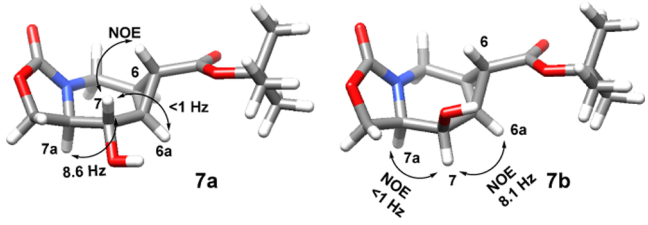
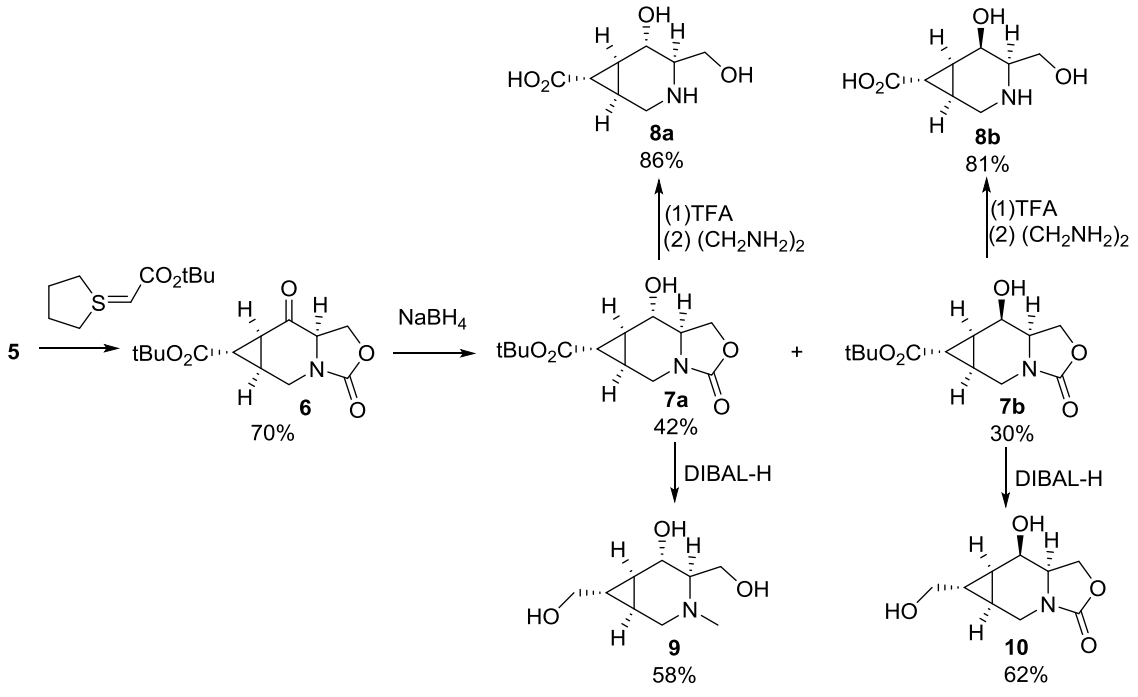


Figure 4. NOE signals and constant couplings in products **7a** and **7b**, respectively.[24]

In continuation of the synthesis, treatment with trifluoroacetyl (TFA) and further reaction with ethylendiamine gave products **8a** and **8b**, respectively, in excellent yields. On the other hand, compounds **7a** and **7b** gave different products on reacting with DIBAL-H, whereas **7b** gave the expected alcohol **10** (62%), additionally, the reaction of **7a** caused the cleavage of the oxazolidinone ring giving **9** in 58% yield. This behavior has been described previously (Scheme 3).[25]

Final compounds were screened for glycosidase inhibition activities (α-glucosidase from *Bacillus stearothermophilus*, β-glucosidase from almonds, α-galactosidase from green coffee beans, β-galactosidase from *Escherichia coli*, α-mannosidase

Scheme 3. Synthesis of Final Compounds from 5



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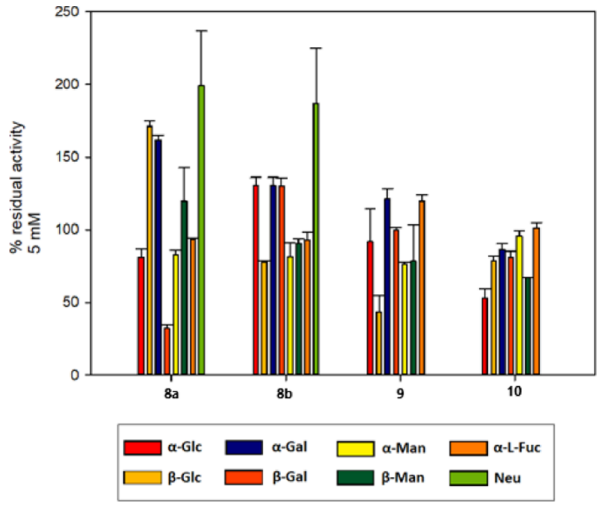
from *Jack beans*, β-mannosidase from *Helix pomatia,* and α-L-fucosidase from *Homo sapiens*) using *p*-nitrophenyl monosaccharides as substrates.

Carboxylic containing compounds **8a** and **8b**, could resemble the zwitterionic form of oseltamivir and zanamivir, well-known inhibitors of neuraminidase from Influenza virus[26] with carboxyl-amino and carboxyl-guanidine moieties, respectively. Preliminary docking calculations using AutoDock[27] showed that **8a** and **8b** could fit in the binding site of neuraminidase. Thus they were also evaluated as possible inhibitors of neuraminidase from *Vibrio cholerae*.

The enzymatic activities were calculated by measuring the absorbance of the phenoxide released in the enzymatic reaction at 405 nm. The compounds were initially screened at 1, 5, and 25 mM concentrations. With compounds **8a** and **9**, inhibition over 50% was observed with selected enzymes at 5 mM; **8b** and **10** did not show any significant inhibition of any glycosidase (Chart 1). Inhibition constants (K_i) were

Chart 1. Residual Glycosidase Activities in the Presence of 5 mM Synthesized Compounds



estimated assuming a competitive type inhibition in the cases of compound **8a** against β-galactosidase (K_i = 1.18 mM) and **9** against β-glucosidase (K_i = 4.43 mM). These two compounds exhibit some selectivity such that even at 25 mM no significant inhibition was observed against other glycosidases.

However, the observed inhibition was very weak compared to other iminosugar-based glycosidase inhibition, for example the measured K_i for deoxinojirimycin (DNJ) is 0.44 μM for α-glucosidase from *B. stearothermophilus*. The inhibition constant changes depending on the species that is studied, even for the same glycosidase of other species.[28] Other iminosugars present great activity against mannosidases.[29]

On the other hand, we found that compounds **8a** and **8b**, bearing a carboxylate group, also did not show any inhibition against neuraminidase but unexpectedly produced activation of the enzyme; these two compounds increased neuraminidase activity up to 100%. The possibility that the compounds act as favorable transglycosylation acceptors causing an increase of nitrophenol release was considered. NMR experiments were performed continuously following the reaction, but potential transient transglycosylation products could not be observed. Further research to explain this behavior is needed. Interestingly there are not many precedents on glycosidase activation by iminosugars. Two reports have accounted for this activation behavior. Thus, up to 70-fold activation of some of glycosidases was detected with multivalent iminosugars.[30] In another study, thienopyrimidines were found to activate certain glycosidases.[31] The activation mechanism could be explained by the stabilization of the active structure of the enzyme by the introduction of a small molecule adjacent/close to the substrate-binding site, locking the reactive form. Alternatively, if the activation is of the allosteric type occurring in a site different from the active site, it could be interesting to check if the activators have any pharmacological chaperone activity but avoid the temporal inhibition of the enzymatic activity, unlike the aforementioned migalastat and other proposed pharmacological chaperones that help maintaining the correct fold of the protein although temporally blocking the active site of the enzyme.

CONCLUSIONS

We described a multigram synthesis of an α,β-unsaturated ketone, which upon a stereoselective cyclopropanation reaction and further transformations gave a novel series of bicyclic piperidine-based iminosugars. The final products were studied against different glycosidases. Inhibition in most cases was low, but interestingly, the activation of neuraminidase was observed with products **8**. Possible explanations of this behavior, for example, allosteric activation, enzyme stabilization, or transglycosylation acceptor activity can be proposed. Current studies in our lab will provide insight into these possible mechanisms, and their potential applications will be explored/pursued.

EXPERIMENTAL SECTION

General Information. All chemicals were obtained from Aldrich/Merck, VWR, Fluorochem, and ABCR. Thin-layer chromatography (TLC) analyses were performed on Merck silica gel 60 F254 plates using phosphomolybdic acid or anisaldehyde and heat for detection. Silica gel NORMASIL 60 40−63 μm was used for flash chromatography. NMR spectra were recorded on a Bruker spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts are reported in δ ppm referenced to $CDCl_3$ (δ = 7.26 for ^{1}H and 77.00 for ^{13}C), CD_3OD (δ = 3.31 for ^{1}H and 49.00 for ^{13}C), or D_2O (δ = 4.79 for ^{1}H). Bidimensional spectra (heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond coherence (HMBC), correlated spectroscopy (COSY), and nuclear Overhauser effect spectroscopy (NOESY)) were recorded in order to carry out the assignment. IR spectra were recorded on a Perkin-Elmer Spectrum 100. Specific optical rotation was measured using a polarimeter Anton Parr MCP 100. Melting points of solid compounds were determined using a Stuart Scientific Melting Point Apparatus SMP3. The absorbance of *p*-nitrophenoxide released in the enzymatic reactions was measured at 405 nm in a Perkin-Elmer Lamba 25.

Methyl (tert-Butoxycarbonyl)-L-serinate. Thionyl chloride (83 mL, 1.1 mol) was added to methanol (280 mL) at 0 °C, then L-serine (60.00 g, 571 mmol) is added. After 10 min, at 0 °C, the solution is heated at 65 °C for 2 h. The solvent is evaporated *in vacuo*, and 600 mL of AcOEt and a saturated solution of $NaHCO_3$ (until basic pH) are added. Di-*tert*-butyl dicarbonate (124.62 g, 0.571 mmol) in 265 mL of AcOEt is added. The reaction is stirred overnight at room temperature.

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The aqueous layer is extracted with AcOEt (2 × 300 mL). The combined organic layers are washed with brine (200 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The crude product is filtered through a pad of silica gel using Hex/AcOEt (9:1) to Hex/AcOEt (3:1) as eluents. A colorless oil is obtained (101.6 g, 81% after two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46 (brs, 1H, NH), 4.39 (brs, 1H, CH), 3.99−3.89 (m, 2H, CH_2O), 3.78 (s, 3H, OMe), 2.47−2.32 (m, 1H, OH), 1.45 (s, 9H, 3 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 155.9, 80.3, 63.3, 55.8, 52.8, 28.3 (3C). IR (NaCl): 3378, 2984, 2868, 1740, 1708 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.13 in dichloromethane (DCM)): +4.14. Found: C, 49.1; H, 7.9%. Calc. for $C_9H_{17}NO_5$: C, 49.3; H, 7.8%.

*tert-Butyl (R)-(1-((tert-Butyldimethylsilyl)oxy)-3-hydroxypropan-2-yl)carbamate (**1**).* To a solution of methyl (*tert*-butoxycarbonyl)-L-serinate (101.4 g, 463 mmol) in 400 mL of dimethylformamide (DMF) cooled to 0 °C is added imidazole (37.8 g, 555 mmol) and 4-dimethylaminopyridine (DMAP; 5.6 g, 46 mmol). After 10 min, *tert*-butyldimethylsilyl chloride (73.2 g, 486 mmol) is added. The reaction is stirred for 30 min at room temperature. AcOEt (400 mL) is added, and the organic layer is washed with water (3 × 1 L) and brine (400 mL), dried over $MgSO_4$, and evaporated *in vacuo*. To a suspension of $NaBH_4$ (35.0 g, 926 mmol) and LiCl (39.3 g, 926 mmol) in 800 mL of ethanol cooled to 0 °C, a solution of the crude in 190 mL of ethanol is added slowly. The reaction is stirred at 0 °C for 10 min, at room temperature for 30 min, and at 50 °C for 2.5 h. The reaction is cooled to 0 °C, and a saturated solution of NH_4Cl is added (until salts are dissolved, 450 mL). The aqueous layer is extracted with AcOEt (3 × 350 mL). The combined organic layers are washed with brine (200 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The reaction crude is filtered through a pad of silica gel using Hex/AcOEt (19:1) to Hex/AcOEt (1:1) as eluents. A colorless oil is obtained (123.2 g, 85% after two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14 (brs, 1H, NH), 3.86−3.66 (m, 5H, 2 × CH_2O + CH), 2.70 (brs, 1H, OH), 1.45 (s, 9H, 3 × CH_3), 0.90 (s, 9H, 3 × CH_3), 0.08 (s, 6H, 2 × CH_3Si). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 79.6, 64.0 (2C), 52.7, 28.5 (3C), 25.9 (3C), 18.3, −5.5 (2C). IR (NaCl): 3371, 2952, 2861, 1707 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.28 in DCM): +9.30. Found: C, 55.1, H, 10.4%. Calc. for $C_{14}H_{31}NO_4Si$: C, 55.0; H, 10.2%.

(R)-4-(((tert-Butyldimethylsilyl)oxy)methyl)oxazolidin-2-one. To a suspension of NaH 60% w/w (18.9 g, 472 mmol) in 300 mL of THF cooled to 0 °C is added a solution of **1** (123.2 g, 403 mmol) in 550 mL of THF. The reaction is stirred at 0 °C for 15 min, at room temperature for 25 min, and at 40 °C for 2.5 h. The reaction is cooled down to 0 °C, and a saturated solution of NH_4Cl is added until all salts are dissolved (250 mL). The aqueous layer is extracted with AcOEt (2 × 500 mL). The combined organic layers are washed with brine (300 mL), dried over $MgSO_4$, and evaporated *in vacuo*. A colorless wax is obtained (78.9 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (brs, 1H, NH), 4.42 (t, *J* = 8.6 Hz, 1H, CH_2O), 4.18 (dd, *J* = 8.8, 4.8 Hz, 1H, CH_2O), 3.94−3.88 (m, 1H, CH), 3.60 (d, *J* = 5.4 Hz, 2H, CH_2OSi), 0.87 (s, 9H, 3 × CH_3), 0.05 (s, 6H, 2 × CH_3Si). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.2, 67.3, 64.8, 53.8, 25.9 (3C), 18.3, −5.4 (2C). IR (NaCl): 3315, 2959, 2848, 1745 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.32 in DCM): −15.94. Found: C, 52.2, H, 9.0%. Calc. for $C_{10}H_{21}NO_3Si$: C, 51.9; H, 9.2%.

*(R)-3-Allyl-4-(((tert-butyldimethylsilyl)oxy)methyl)-oxazolidin-2-one (**2**).* To a suspension of NaH 60% w/w (16.4 g, 409.1 mmol) in 500 mL of THF at 0 °C, a solution of (*R*)-4-(((*tert*-butyldimethylsilyl)oxy)methyl)oxazolidin-2-one (78.9 g, 340.9 mmol) in 500 mL of THF is added slowly. Allyl bromide (29.5 mL, 340.9 mmol) is added and stirred for 15 min at 0 °C, 30 min at room temperature, and 2 h at 50 °C. A saturated solution of NH_4Cl is added until the salts are dissolved. The aqueous layer is extracted with AcOEt (3 × 200 mL). The combined organic layers are washed with brine (150 mL) and dried over $MgSO_4$. The solvent is evaporated *in vacuo*, and the residue is filtered through a pad of silica gel using Hex/AcOEt (19:1) to Hex/AcOEt (1:1) as eluents. A yellow oil is obtained (64.8 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84−5.74 (m, 1H, HC═), 5.27−5.21 (m, 2H, H_2C═), 4.33 (t, *J* = 8.7 Hz, 1H, CH_2O), 4.18−4.13 (m, 2H, CH_2O + CH_2N), 3.86−3.80 (m, 1H, CH), 3.69−3.62 (m, 3H, CH_2OSi + CH_2N), 0.89 (s, 9H, 3 × CH_3), 0.06 (s, 6H, 2 × CH_3Si). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.4, 132.7, 118.5, 65.0, 62.2, 56.0, 45.3, 25.9 (3C), 18.3, −5.4 (2C). IR (NaCl): 3084, 2948, 2866, 1744 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.21 in $CHCl_3$): −11.84. Found: C, 57.1, H, 9.1%. Calc. for $C_{13}H_{25}NO_3Si$: C, 57.5; H,9.3%.

(S)-3-Allyl-4-(hydroxymethyl)oxazolidin-2-one. To a solution of **2** (55.5 g, 204.6 mmol) in 220 mL of THF is added TBAF·$3H_2O$ (58.8 g, 225.0 mmol). The mixture is stirred for 30 min at room temperature. The solvent is evaporated *in vacuo* and filtered through a pad of silica gel using Hex/AcOEt (2:1) to Hex/AcOEt (1:2) as eluents. A colorless oil is obtained (28.9 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86−5.76 (m, 1H, HC═), 5.30−5.24 (m, 2H, H_2C═), 4.36 (t, *J* = 8.8 Hz, 1H, CH_2O), 4.25 (dd, *J* = 8.7, 6.0 Hz, 1H, CH_2O), 4.09 (ddt, *J* = 15.7, 5.3, 1.6 Hz, 1H, CH_2N), 3.90−3.84 (m, 1H, CHN), 3.80−3.73 (m, 2H, CH_2N + CH_2OH), 3.65 (dd, *J* = 11.9, 3.3 Hz, 1H, CH_2OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 132.5, 118.9, 64.7, 60.9, 56.3, 45.4. IR (NaCl): 3427, 3048, 2975, 2851, 1753 cm^{-1}. $[\alpha]_D^{25}$ (0.25 in $CHCl_3$): −44.28. Found: C, 53.8; H, 7.3%. Calc. for $C_7H_{11}NO_3$: C, 53.5; H, 7.1%.

*(R)-3-Allyl-2-oxo-oxazolidine-4-carboxylic Acid (**3**).* A solution of (*S*)-3-allyl-4-(hydroxymethyl)oxazolidin-2-one (28.9 g, 184.1 mmol) in 1 L of acetone is cooled to 0 °C, and 92 mL of Jones' reagent is added slowly. The reaction is stirred for 1.5 h at 0 °C. Isopropanol is added until the solution turns blue. The mixture is filtered through a pad of celite. The solvent is evaporated *in vacuo* and the residue is filtered through a pad of silica gel using Hex/AcOEt (1:1) to AcOEt 100% as eluents. A pale yellow oil is obtained (23.0 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (brs, 1H, OH), 5.80−5.70 (m, 1H, HC═), 5.27−5.24 (m, 2H, H_2C═), 4.52 (t, *J* = 9.4 Hz, 1H, CH_2O), 4.42 (dd, *J* = 9.0, 4.5 Hz, 1H, CH_2O), 4.36 (dd, *J* = 9.7, 4.6 Hz, 1H, CHN), 4.27 (dd, *J* = 15.4, 4.8 Hz, 1H, CH_2N), 3.75 (dd, *J* = 15.4, 8.0 Hz, 1H, CH_2N). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 158.5, 131.0, 120.1, 65.1, 56.0, 46.0. IR (NaCl): 3454, 2933, 2839, 1731 cm^{-1}. $[\alpha]_D^{25}$ (0.30 in $CHCl_3$): +8.98. Found: C, 48.8; H, 5.4%. Calc. for $C_7H_9NO_4$: C, 49.1; H, 5.3%.

*(R)-3-Allyl-N-methoxy-N-methyl-2-oxo-oxazolidine-4-carboxamide (**4**).* A solution of **3** (23.0 g, 134.4 mmol) in 500 mL of DCM is cooled to 0 °C. Diisopropylethylamine (DIPEA; 23.5 mL, 134.4 mmol), EDCI (25.8 g, 134.4 mmol), and *N*,*O*-dimethylhydroxylamine hydrochloride (13.1 g, 134.4 mmol) are added. The reaction is stirred for 2 h at 0 °C. The solvent is evaporated *in vacuo* and filtered through a pad of silica gel using Hex/AcOEt (1:4) as the eluent. A yellow oil is obtained (25.3 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83−5.73 (m,

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1H, HC═), 5.25−5.21 (m, 2H, H_2C═), 4.66 (dd, *J* = 9.8, 5.6 Hz, 1H, CHN), 4.50 (t, *J* = 9.3 Hz, 1H, CH_2O), 4.32 (ddt, *J* = 15.4, 4.7, 1.7 Hz, 1H, CH_2N), 4.18 (dd, *J* = 8.8, 5.6 Hz, 1H, CH_2O), 3.69 (s, 3H, OCH_3), 3.68 (dd, *J* = 15.4, 8.3 Hz, 1H, CH_2N), 3.22 (s, 3H, NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 158.0, 132.2, 119.3, 64.5, 61.7, 54.8, 45.9, 32.7. IR (NaCl): 3088, 2979, 2928, 1754, 1672 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.37 in DCM): +31.11. Found: C, 50.4; H, 6.9%. Calc. for $C_9H_{14}N_2O_4$: C, 50.5; H, 6.6%.

(R)-4-Acryloyl-3-allyloxazolidin-2-one. To a solution of **4** (12.0 g, 56.0 mmol) in 270 mL of THF cooled to −30 °C, 0.7 M vinylmagnesium bromide (200 mL) is added slowly, keeping the temperature below −25 °C. When the addition is finished, the reaction is stirred for another 30 min at −30 °C. The reaction mixture is poured into a mixture of 200 mL of HCl 10% and 100 mL of MeOH cooled in a bath at −15 °C. This mixture is stirred for another 15 min. The aqueous layer is extracted with AcOEt (3 × 150 mL). The combined organic layers are washed with a solution of 1 M HCl (200 mL), with a saturated solution of $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The crude is used without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 6.50 (dd, *J* = 17.5, 10.4 Hz, 1H, ═CHCO), 6.39 (d, *J* = 17.4 Hz, 1H, H_2C ═ CHCO, trans), 6.01 (d, *J* = 10.4 Hz, 1H, H_2C ═ CHCO, cis), 5.78−5.68 (m, 1H, HC═), 5.24−5.16 (m, 2H, H_2C═), 4.59 (dd, *J* = 10.0, 5.2 Hz, 1H, CHN), 4.52 (t, *J* = 9.3 Hz, 1H, CH_2O), 4.26 (dd, *J* = 15.3, 4.6 Hz, 1H, CH_2N), 4.15 (dd, *J* = 8.6, 5.2 Hz, 1H, CH_2O), 3.59 (dd, *J* = 15.3, 8.0 Hz, 1H, CH_2N). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.1, 157.7, 132.3, 131.8, 131.4, 120.1, 63.8, 60.5, 46.2.

*(R)-1,8a-Dihydro-3H-oxazolo[3,4-a]pyridine-3,8(5H)-dione (**5**).* The crude of the previous reaction is dissolved in 180 mL of DCM and heated to reflux. Grubb's second-generation catalyst is added (1.2 g, 1.4 mmol). The reaction is stirred under reflux for 1.5 h. The solvent is evaporated *in vacuo*. The crude is purified in silica gel in Hex/AcOEt (1:1). A brown oil is obtained (5.6 g, 65% after two steps). ^1H NMR (400 MHz, $CDCl_3$) δ 7.06 (ddd, *J* = 10.5, 4.4, 2.0 Hz, 1H, ═ C**H**CH_2), 6.26 (dt, *J* = 10.5, 2.3 Hz, 1H, ═CHCO), 4.69 (dd, *J* = 9.0, 4.4 Hz, 1H, CHN), 4.61−4.50 (m, 2H, CH_2O + CH_2N), 4.27 (ddd, *J* = 9.4, 4.4, 2.1 Hz, 1H, CH_2O), 4.02 (dq, *J* = 20.5, 2.3 Hz, 1H, CH_2N). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.1, 157.4, 146.5, 127.7, 64.2, 57.9, 41.8. IR (NaCl): 3081, 2959, 2920, 2854, 1752, 1748 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.09 in DCM): +65.02. Found: C, 55.1; H, 4.5%. Calc. for $C_7H_7NO_3$: C, 54.9; H, 4.6%.

*tert-Butyl (5aR,6S,6aS,7aR)-3,7-Dioxohexahydro-1H,3H-cyclopropa[d]oxazolo[3,4-a]pyridine-6-carboxylate (**6**).* To a solution of **5** (2.4 g, 15.9 mmol) in 13 mL of DCM at 0 °C is added a solution of *tert*-butyl (tetrahydrothiophenylidene)-acetate (9.6 g, 47.6 mmol) in 207 mL of DCM slowly. The reaction is stirred at room temperature for 30 min. Deionized water (20 mL) is added. The aqueous layer is extracted with DCM (2 × 30 mL). The combined organic phases are washed with brine (20 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The crude is purified in silica gel using Hex/AcOEt (2:1) as the eluent. A yellow wax is obtained (3.0 g, 70%). ^1H NMR (400 MHz, $CDCl_3$) δ 4.53 (t, *J* = 9.7 Hz, 1H, CH_2O), 4.32 (dd, *J* = 9.3, 5.7 Hz, 1H, CH_2O), 4.23 (d, *J* = 14.1 Hz, 1H, CH_2N), 4.04 (dd, *J* = 10.3, 5.7 Hz, 1H, CHN), 3.53 (d, *J* = 14.0 Hz, 1H, CH_2N), 2.39 (dd, *J* = 7.9, 4.2 Hz, 1H, CHCO), 2.21−2.18 (m, 1H, C**H**CH_2N) 2.10 (t, *J* = 4.5 Hz, 1H, $CHCO_2$), 1.44 (s, 9H, 3 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.0, 168.9, 156.9, 82.8, 64.2, 58.8, 37.2, 31.6, 28.1, 24.5, 22.7. IR (NaCl): 2975, 2863, 1748, 1736, 1719 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.11 in DCM): +38.02. Found: C, 58.0; H, 4.9%. Calc. for $C_{13}H_{17}NO_5$: C, 58.4; H, 4.6%.

*tert-Butyl (5aR,6S,6aS,7S,7aR) and tert-butyl (5aR,6-S,6aS,7R,7aR)-7-Hydroxy-3-oxohexahydro-1H,3H-cyclopropa[d]oxazolo[3,4-a]pyridine-6-carboxylate (**7a** and **7b**).* To a solution of **6** (1.1 g, 4.1 mmol) in 35 mL of absolute ethanol at 0 °C is added $NaBH_4$ (312 mg, 8.2 mmol). The reaction is stirred for 1 h at room temperature. A solution of saturated NH_4Cl (20 mL) and water (until salts dissolve) is added. The aqueous phase is extracted with AcOEt (3 × 60 mL). The combined organic layers are washed with brine (50 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The crude contained a 3:2 mixture of isomers **7a**/**7b** as determined by the integration of signals in the ^1H NMR spectrum of the reaction crude. This mixture was separated by silica gel chromatography using Hex/AcOEt (1:1) to Hex/AcOEt (1:2) as eluents. A yellow wax is obtained for isomer **7a** (463 mg, 42%). A yellow solid is obtained for isomer **7b** (330 mg, 30%).

Spectroscopic data for *tert*-butyl (5a*R*,6*S*,6a*S*,7*S*,7a*R*)-7-hydroxy-3-oxohexahydro-1*H*,3*H*-cyclopropa[*d*]oxazolo[3,4-*a*]-pyridine-6-carboxylate **7a**: ^1H NMR (400 MHz, $CDCl_3$) δ 4.52 (t, *J* = 8.6 Hz, 1H, CH_2O), 4.12 (dd, *J* = 9.1, 4.8 Hz, 1H, CH_2O), 4.00 (d, *J* = 13.6 Hz, 1H, CH_2N), 3.80 (dd, *J* = 8.5, 4.5 Hz, 1H, C**H**OH), 3.40 (dd, *J* = 13.6, 4.1 Hz, 1H, CH_2N), 3.33 (td, *J* = 8.3, 4.9 Hz, 1H, CHN), 2.41 (d, *J* = 5.0 Hz, 1H, OH), 1.76−1.70 (m, 1H, C**H**CH_2N), 1.67 (dd, *J* = 9.1, 5.0 Hz, 1H, C**H**CHOH), 1.44 (s, 9H, 3 × CH_3), 1.40 (t, *J* = 4.9 Hz, 1H, $CHCO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 157.5, 81.4, 69.0, 68.3, 56.8, 38.5, 28.2 (3C), 27.3, 24.5, 20.3. IR (NaCl): 3361, 2975, 2863, 1748, 1736 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.14 in DCM): −2.20. Found: C, 58.2; H, 6.8%. Calc. for $C_{13}H_{19}NO_5$: C, 58.0; H, 7.1%.

Spectroscopic data for *tert*-butyl (5a*R*,6*S*,6a*S*,7*R*,7a*R*)-7-hydroxy-3-oxohexahydro-1*H*,3*H*-cyclopropa[*d*]oxazolo[3,4-*a*]-pyridine-6-carboxylate **7b**: ^1H NMR (400 MHz, $CDCl_3$) δ 4.54 (dd, *J* 0 8.5, 6.4 Hz, 1H, CH_2O), 4.29 (t, *J* = 8.8 Hz, 1H, CH_2O), 4.24−4.20 (m, 1H, C**H**OH), 4.00 (d, *J* = 13.4 Hz, 1H, CH_2N), 3.61−3.55 (m, 1H, CHN), 3.29 (dd, *J* = 13.4, 4.0 Hz, 1H, CH_2N), 2.11 (td, *J* = 8.4, 5.0 Hz, 1H, C**H**CHOH), 1.97 (d, *J* = 4.1 Hz, 1H, OH), 1.84−1.74 (m, 2H, 2 × CH cyclopropane), 1.44 (s, 9H, 3 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 157.8, 81.5, 63.1, 60.4, 55.7, 38.8, 28.3 (3C), 25.9, 21.2, 20.5. IR (KBr): 3361, 2975, 2863, 1748, 1736 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.04 in DCM): −10.46. Found: C, 58.3; H, 7.0%. Calc. for $C_{13}H_{19}NO_5$: C, 58.0; H, 7.1%. Mp > 180.0 °C, dec.

(5aR,6S,6aS,7S,7aR)-7-Hydroxy-3-oxohexahydro-1H,3H-cyclopropa[d]oxazolo[3,4-a]pyridine-6-carboxylic Acid. To a solution of **7a** (250 mg, 0.9 mmol) in 1.5 mL of DCM is added 9.3 mL of TFA at room temperature. The reaction is stirred for 30 min. The solvent is evaporated *in vacuo*. Toluene is added until all TFA is evaporated. The crude is purified in silica gel in 10% DCM/MeOH. A brown wax is obtained (187 mg, 94%). ^1H NMR (400 MHz, MeOD) δ 4.54 (t, *J* = 8.5 Hz, 1H, CH_2O), 4.14 (dd, *J* = 8.9, 5.2 Hz, 1H, CH_2O), 3.87 (d, *J* = 13.6 Hz, 1H, CH_2N), 3.82 (d, *J* = 8.7 Hz, 1H, C**H**OH), 3.44 (dd, *J* = 13.5, 4.2 Hz, 1H, CH_2N), 3.37 (td, *J* = 8.5, 5.2 Hz, 1H, CHN), 1.84−1.75 (m, 1H, C**H**CH_2N), 1.70 (dd, *J* = 9.3, 4.7 Hz, 1H, C**H**CHOH), 1.38 (brs, 1H, $CHCO_2$). ^{13}C NMR (100 MHz, D_2O) δ 176.6, 159.5, 69.8, 69.6, 58.2, 39.5, 29.0, 24.5, 21.9. IR (NaCl): 3396, 2984, 2851, 1748, 1729 cm^{-1}. $[\alpha]_D^{25}$ (*c*

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0.05 in MeOH): −3.68. Found: C, 51.1; H, 5.3%. Calc. for $C_9H_{11}NO_5$: C, 50.7; H, 5.2%.

(5aR,6S,6aS,7R,7aR)-7-Hydroxy-3-oxohexahydro-1H,3H-cyclopropa[d]oxazolo[3,4-a]pyridine-6-carboxylic Acid. To a solution of **7b** (86 mg, 0.3 mmol) in 1 mL of DCM is added 3.2 mL of TFA at room temperature. The reaction is stirred for 30 min. The solvent is evaporated *in vacuo*. Toluene is added until all TFA is evaporated. The crude is purified in silica gel in 10% DCM/MeOH. A brown wax is obtained (60 mg, 90%). ^{1}H NMR (400 MHz, D_2O) δ 4.47 (dd, J = 8.5, 5.8 Hz, 1H, CH_2O), 4.31 (t, J = 8.8 Hz, 1H, CH_2O), 4.17 (dd, J = 8.0, 3.8 Hz, 1H, C**H**OH), 3.86 (d, J = 13.4 Hz, 1H, CH_2N), 3.71 (ddd, J = 9.5, 5.9, 3.9 Hz, 1H, CHN), 3.38 (dd, J = 13.4, 4.4 Hz, 1H, CH_2N), 2.09 (td, J = 8.4, 4.7 Hz, 1H, C**H**CHOH), 1.82 (t, J = 4.9 Hz, 1H, $CHCO_2$), 1.80−1.75 (m, 1H, C**H**CH_2N). ^{13}C NMR (100 MHz, D_2O) δ: 177.7, 159.6, 64.2, 59.8, 55.4, 38.4, 26.0, 21.2, 20.0. IR (KBr): 3388, 2991, 2867, 1740, 1732 cm^{-1}. $[\alpha]_D^{25}$ (c 0.02 in MeOH): −48.87. Found: C, 51.0; H, 4.9%. Calc. for $C_9H_{11}NO_5$: C, 50.7; H, 5.2%. Mp > 205.4 °C, dec.

*(1R,4R,5S,6S,7S)-5-Hydroxy-4-(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-7-carboxylic Acid (**8a**).* To a solution of (5a*R*,6*S*,6a*S*,7*S*,7a*R*)-7-hydroxy-3-oxohexahydro-1*H*,3*H*-cyclopropa[*d*]oxazolo[3,4-*a*]pyridine-6-carboxylic acid (187 mg, 0.9 mmol) in 2 mL of MeOH is added ethylenediamine (0.18 mL, 2.6 mmol) at room temperature and heated at 60 °C for 1.5 h. The solvent is evaporated *in vacuo* and methanol is added to evaporate excess amine. A solution of HCl 4 N in dioxane (5 mL) is added and stirred for 30 min. A yellow wax is obtained (150 mg, 91%).^{1}H NMR (400 MHz, MeOD) δ 4.54 (t, J = 8.5 Hz, 1H, CH_2O), 4.14 (dd, J = 8.9, 5.3 Hz, 1H, CH_2O), 3.86 (d, J = 13.5 Hz, 1H, CH_2N), 3.72 (d, J = 8.7 Hz, 1H, C**H**OH), 3.44 (dd, J = 13.5, 4.3 Hz, 1H, CH_2N), 3.36 (td, J = 8.4, 5.2 Hz, 1H, CHN), 1.78−1.70 (m, 1H, C**H**CH_2N), 1.67 (dd, J = 9.2, 4.9 Hz, 1H, C**H**CHOH), 1.32 (t, J = 4.9 Hz, 1H, $CHCO_2$). ^{13}C NMR (100 MHz, MeOD) δ 159.5, 70.1, 69.6, 58.2, 39.6, 28.5, 25.7, 21.3. IR (NaCl): 3405, 2996, 2895, 1736 cm^{-1}. $[\alpha]_D^{25}$ (c 0.05 in MeOH): −5.41. Found: C, 51.0; H, 7.2%. Calc. for $C_8H_{13}NO_4$: C, 51.3; H, 7.0%.

*(1R,4R,5R,6S,7S)-5-Hydroxy-4-(hydroxymethyl)-3-azabicyclo [4.1.0]heptane-7-carboxylic Acid (**8b**).* To a solution of (5a*R*,6*S*,6a*S*,7*R*,7a*R*)-7-hydroxy-3-oxohexahydro-1*H*,3*H*-cyclopropa[*d*]oxazolo[3,4-*a*]pyridine-6-carboxylic acid (75 mg, 0.4 mmol) in 1 mL of MeOH is added ethylenediamine (0.07 mL, 1.1 mmol) and heated at 60 °C for 1.5 h. The solvent is evaporated *in vacuo* and methanol is added to evaporate excess amine. A solution of HCl 4 N in dioxane (1 mL) is added and stirred for 30 min. A yellow wax is obtained (60 mg, 90%). ^{1}H NMR (400 MHz, MeOD) δ 4.47 (dd, J = 8.5, 6.0 Hz, 1H, CH_2O), 4.30 (t, J = 8.8 Hz, 1H, CH_2O), 4.16 (dd, J = 8.0, 3.9 Hz, 1H, C**H**OH), 3.84 (d, J = 13.3 Hz, 1H, CH_2N), 3.75−3.62 (m, 1H, CHN), 3.37 (dd, J = 13.2, 4.4 Hz, 1H, CH_2N), 2.11−1.93 (m, 1H, C**H**CHOH), 1.79−1.64 (m, 2H, 2 × CH cyclopropane). ^{13}C NMR (100 MHz, MeOD) δ 158.80, 63.53, 59.64, 55.65, 38.46, 25.01, 21.50, 19.60. IR (NaCl): 3402, 2984, 2890, 1733 cm^{-1}. $[\alpha]_D^{25}$ (c 0.02 in MeOH): −32.0. Found: C, 51.4; H, 7.2%. Calc. for $C_8H_{13}NO_4$: C, 51.3; H, 7.0%.

*(1S,4R,5S,6S,7S)-4,7-bis(Hydroxymethyl)-3-methyl-3-azabicyclo[4.1.0]heptan-5-ol (**9**).* To a solution of **7a** (120 mg, 0.5 mmol) in 3 mL of DCM is added 1.86 mL of DIBAL-H 1,2M in toluene at 0 °C. The reaction is stirred for 4 h at room temperature. Methanol is added (10 mL). The salts are filtered and rinsed with methanol (2 × 10 mL). The solvent is evaporated *in vacuo*. The crude is purified in silica gel using MeCN/H_2O (9:1) as the eluent. A yellow wax is obtained (49 mg, 58%). ^{1}H NMR (400 MHz, MeOD) δ 3.81 (dd, J = 11.7, 3.2 Hz, 1H, CH_2O), 3.77−3.71 (m, 2H, CH_2O + C**H**OH), 3.45 (dd, J = 11.3, 6.7 Hz, 1H; HOC**H**$_2$Ccyclopropane), 3.37 (dd, J = 11.3, 6.8 Hz, 1H, HOC**H**$_2$Ccyclopropane), 3.07 (d, J = 11.7 Hz, 1H, CH_2N), 2.63 (dd, J = 11.6, 3.9 Hz, 1H, CH_2N), 2.38 (s, 3H, NCH_3), 1.75 (dt, J = 7.9, 2.9 Hz, 1H, CHN), 1.23−1.15 (m, 1H, HOCH_2C**H**cyclopropane), 1.12−1.03 (m, 1H, C**H**CH_2N), 0.99 (dd, J = 9.0, 4.6 Hz, 1H, C**H**CHOH). ^{13}C NMR (100 MHz, MeOD) δ 70.2, 67.1, 65.9, 60.1, 55.6, 43.0, 23.24, 23.22, 17.3. IR (NaCl): 3357, 2993, 2892 cm^{-1}. $[\alpha]_D^{25}$ (c 0.03 in MeOH): −4.65. Found: C, 57.5, H, 9.5%. Calc. for $C_9H_{17}NO_3$: C, 57.7; H, 9.2%.

*(5aS,6S,6aS,7R,7aR)-7-Hydroxy-6-(hydroxymethyl)-hexahydro-1H,3H-cyclopropa[d]oxazolo[3,4-a]pyridin-3-one (**10**).* To a solution of **7b** (117 mg, 0.4 mmol) in 3 mL of DCM at 0 °C is added 1.80 mL of 1,2M DIBAL-H in toluene. The reaction is stirred for 4 h at room temperature. Methanol is added (10 mL). The salts are filtered and rinsed with methanol (2 × 10 mL). The solvent is evaporated *in vacuo*. The crude is purified in silica gel using MeCN as the eluent. A yellow wax is obtained (54 mg, 62%). ^{1}H NMR (400 MHz, MeOD) δ 4.48 (dd, J = 8.5, 5.8 Hz, 1H, CH_2O), 4.32 (t, J = 8.8 Hz, 1H, CH_2O), 4.16 (dd, J = 8.1, 3.8 Hz, 1H, C**H**OH), 3.83 (d, J = 12.9 Hz, 1H, CH_2N), 3.69 (ddd, J = 9.4, 5.8, 3.8 Hz, 1H, CHN), 3.54 (dd, J = 11.2, 6.4 Hz, 1H, HOC**H**$_2$Ccyclopropane), 3.42 (dd, J = 11.3, 6.7 Hz, 1H, HOC**H**$_2$Ccyclopropane), 3.36 (dd, 12.9, 4.7 Hz, 1H, CH_2N), 1.42 (td, J = 8.4, 4.9 Hz, 1H, C**H**CHOH), 1.30−1.20 (m, 1H, HOCH_2C**H** cyclopropane), 1.19−1.09 (m, 1H, C**H**CH_2N). ^{13}C NMR (100 MHz, MeOD) δ 160.4, 65.6, 65.0, 61.4, 57.2, 40.2, 21.9, 21.0, 16.5. IR (NaCl): 3384, 2991, 2888, 1705 cm^{-1}. $[\alpha]_D^{25}$ (c 0.01 in MeOH): −35.3. Found: C, 54.5; H, 6.5%. Calc. for $C_9H_{13}NO_4$: C, 54.3; H, 6.6%.

General Procedure for Enzymatic Reactions. Glycosidase activities were assessed in 80 μL reaction volumes in Eppendorf vials. Buffer composition and enzyme concentration were adjusted depending on the enzyme assayed: 20 mM Na_2HPO_4 at pH 7.3 for β-glucosidase (3 μg/mL) and β-galactosidase (1 μg/mL); 20 mM Na_2HPO_4 at pH 6.8 for α-glucosidase (1 μg/mL) and α-galactosidase (20 μM); 20 mM NaH_2PO_4 at pH 5.5 for α- and β-mannosidase (7 and 2 μM respectively); 0.1 M NaOAc at pH 4.0 with 1 mg/mL of bovine serum albumin (BSA) for α-L-fucosidase (2 μM); and 50 mM NaOAc at pH 5.0 for neuraminidase (6 μM). The inhibitors were tested at 1, 5, and 25 mM final concentrations in the assays. Each enzyme mixture and inhibitor were homogenized and preincubated for 10 min at 37 or 40 °C (α-L-fucosidase). Each reaction was initiated and brought to a final volume of 80 μL, by addition of an aliquot of the corresponding *p*-nitrophenyl glycoside substrate to obtain the following final concentrations in the reaction mixtures: *p*-nitrophenyl α- and β-D-glucopyranoside (1 mM), *p*-nitrophenyl α- and β-D-galactopyranoside (0.5 mM), *p*-nitrophenyl α- and β-D-mannopyranoside (1 mM), *p*-nitrophenyl α-L-fucopyranoside (1 mM), or *p*-nitrophenyl neuraminic acid (1 mM). After 10 min of incubation time at the same temperature, each reaction was quenched with 400 μL of 1.0 M Na_2CO_3, and the absorbance at 405 nm was measured. Assays were repeated twice and data were averaged.

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The residual activity of each enzyme was calculated by the ratio of the absorbance measured after 10 min of reaction in the presence and absence of synthesized compounds. The equation used to calculate K_i was derived from Michaelis−Menten, where V_i is the absorbance measured in the absence of the synthesized compounds; V is the absorbance when the compounds were added to the enzymatic reaction; K_m indicates the Michaelis−Menten constant for each enzyme; and [I] is the concentration of the synthesized compounds (5 mM) and [S] is the concentration of the substrate (eq 1).

Calculated K_i for compounds 8a and 9

$$K_i = \frac{K_m[I]}{(K_m + [S])(V/V_i - 1)} \quad (1)$$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04589.

^{1}H NMR, ^{13}C NMR, and IR spectra of synthesized compounds (PDF)

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Notes

The authors declare no competing financial interest.

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